Expression Analysis Using Oligonucleotide Microarrays in Mice Lacking Bradykinin Type 2 Receptors

Jan Monti, Volkmar Gross, Friedrich C. Luft, Anna Franca Milia, Herbert Schulz, Rainer Dietz, Arya M. Sharma, Norbert Hübner

Abstract—We recently conducted detailed cardiovascular and blood pressure–related phenotypic studies of mice lacking the bradykinin-B2 receptor and were unable to identify a phenotype despite insensitivity to infused bradykinin. We therefore used oligonucleotide microarray analysis of some 12 000 genes and expressed sequence tags to identify molecular mechanisms that might be involved in compensating for the lack of a functional B2 receptor in the kidneys of the mice. We identified 2 gene families that may have an impact on cardiovascular regulation and the bradykinin pathway. A water transport channel in the kidney, AQP4, was downregulated in the mice, whereas other members of the gene family did not show differences in expression levels. In addition, a number of serine proteases were upregulated in B2 receptor–deficient mice. These genes are all located within a gene cluster on mouse chromosome 7. The findings were verified by an independent method. We suggest that microarray analysis has usefulness in elucidating otherwise unappreciated compensatory signaling pathways. (Hypertension. 2001;38:e1-e3.)

Key Words: bradykinin genes chromosome 7 microarray analysis

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inins are important mediators of cardiovascular homeostasis, as well as inflammation and nociception. Bradykinin, the major effector peptide of the kallikrein-kinin system, induces vasodilation and natriuresis-diuresis and influences cardiovascular structure.1 Mice lacking a functional B2 receptor have elevated baseline blood pressure levels and exhibit salt-sensitive hypertension according to earlier reports.2,3 We embarked on comprehensive physiological studies to define the mechanisms of these findings. However, with telemetry, detailed renal physiology, and cardiac morphology, we were unable to replicate the earlier findings.4 Given that pathophysiological findings in B2 receptor–deficient mice derived from the original stock could not be reproduced, we hypothesized that differences in the genetic background may account for the lack of perturbances in blood pressure homeostasis and pressure natriuresis-diuresis in these mice. Because absence of a functional B2 receptor was confirmed both structurally and pharmacologically in these studies,4 we concluded that the absence of blood pressure differences between B2 receptor–deficient and 129Sv/J mice might indicate important, yet unappreciated, physiological adjustments occurring in these mice. To test this notion, we used oligonucleotide microarray analysis of some 12 000 genes and expressed sequence tags to identify molecular mechanisms underlying physiological processes compensating the lack of a functional B2 receptor.

Parallel analysis of gene expression was performed with commercial mouse gene probe arrays with the capacity to display transcript levels of approximately 12 000 mouse genes and expressed sequence tags (U74A, Affymetrix). B2 receptor–deficient mice were obtained from breeder pairs supplied by the Department of Pharmacology, University of Sassari, Italy, and are described elsewhere.2 129Sv/J mice were derived from breeder pairs from the Jackson Laboratory (Bar Harbor, Me). Both strains were propagated by strict inbreeding in our animal facility and kept under specific pathogen-free conditions. All experiments were conducted in male mice 16 weeks of age and involved 2 mice per study group for microarray analysis performed according to the supplier’s instructions.5

The number of differentially expressed genes observed in comparisons between B2 receptor–deficient mice and controls clearly exceeded the variance in expression due to target preparation and independent sampling from animals within the same group, with specific correlations of $r^2=0.974$ for 129Sv/J control versus control and $r^2=0.971$ for B2 receptor–deficient mice. Our analysis revealed 4302 genes scored present in B2 receptor–deficient mice compared with 4498 expressed genes in 129Sv/J mice. A total of 3940 genes were present in all 4 independent hybridization experiments. Shown in the Figure is a schematic representation of all genes that received an increased or decreased call in 2 replicated
Differentially expressed genes in between mice lacking the B2 receptor and controls. Gene expression patterns in B2 receptor–deficient and control mice generated from 2 independent mice per group. Bars represent the fold change of transcript levels of a particular gene when comparing B2 receptor–deficient and control mice. These differentially expressed genes were grouped into classes according to function and upregulation or downregulation.

We identified 2 gene families that might have an impact on cardiovascular regulation and the bradykinin pathway. We discovered the differential regulation of a water transport channel in the kidney, AQP4, and confirmed that other members of the gene family did not show any difference in their expression level, as shown in the Figure. Another member of the aquaporin gene family, AQP1, showed a reduced abundance of transcript in mice lacking the B2 receptor (20% reduction). Although the decreased expression was not formally called by the analysis software, we believe that the difference is real in view of the very high expression level of this gene in the kidney. Aquaporins play an important role in the transport of water across membranes. Although we observed a specific deregulation of AQP1 and AQP4 expression, the transcriptional level of additional members of this gene family, such as AQP7 and AQP8, remained unchanged, when comparing B2 receptor–deficient mice and controls. Both AQP1 and AQP4 are expressed in the proximal tubule in mice. Interestingly, a synergistic effect defect in urinary concentrating capacity of AQP1 and AQP4 has recently been proposed in double knockout animals. We can only speculate whether or not adjustments in AQP1 and AQP4 could influence blood pressure or account for absence of the B2 receptor. However, antidiuretic hormone-related mechanisms have been suggested to influence blood pressure regulation in numerous earlier studies reviewed elsewhere. In addition, a number of serine proteases were found to be specifically upregulated in B2 receptor–deficient mice. This observation suggests that a feedback mechanism exists between the absence of the B2 receptor and enzymes involved in the processing of high molecular weight kinogen to bradykinin. Interestingly, these genes are all located within a gene cluster on mouse chromosome 7. Thus, a common pathway may regulate their expression.

In a subsequent study, we confirmed the differential gene expression between B2 receptor–deficient mice (n=5) and controls (n=6) for prostate-specific antigen, kallikrein, AQP4, and AQP1 by an independent method using real-time polymerase chain reaction, with fold change factors of 2.5, P<10^{-5}; 1.9, P<0.01; −4.5, P<10^{-5}; and −1.2, P<0.05, respectively. In summary, we performed a gene expression analysis in the kidneys of B2 receptor–deficient and control mice to explain our earlier findings that could not substantiate cardiovascular phenotypes in mice lacking the B2 receptor. We found differences in gene expression between B2 receptor–deficient and control mice, indicating adaptive mechanisms to the lack of a functional B2 receptor. Our analysis indicates that those related to aquaporin expression may be of relevance to our physiological findings, and we believe that systematic gene expression studies will help elucidating unappreciated physiological mechanisms.

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